

# The DFRC Method for Lignin Analysis: The Behavior of Cinnamyl End-groups

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## Introduction

Lignins are complex natural polymers arising from an enzyme-mediated dehydrogenative polymerization of phenylpropanoid precursors, primarily coniferyl and sinapyl alcohols. Softwood guaiacyl lignins are derived primarily from coniferyl alcohol whereas hardwood and non-woody plant (e.g. forage grasses and legumes) guaiacyl-syringyl lignins come from a mixture of coniferyl and sinapyl alcohols. Lignification involves free-radical coupling reactions, sometimes combined with nucleophilic additions to quinone methide intermediates, to form three-dimensional polymers. Sarkanen and Ludwig's seminal Lignin book reviewed the two types of polymerization processes for synthetic lignin (DHP) formation *in vitro* originated by Bernd Lehman and John M. Harkin (Harkin, personal communication). The 'Zutropf' DHPs, formed by adding lignin precursors slowly and continuously, were called 'end-wise' polymers and structurally resembled isolated lignins more closely than 'Zulauf' DHPs or 'bulk' polymers, formed by adding the precursors in a single batch.

One characteristic difference between end-wise and bulk synthetic lignin polymers is that there are fewer cinnamyl end-groups in the former than in the latter, because bulk lignification involves substantial immediate dimerization. End-wise polymerization more frequently involves addition of a monomer to a growing lignin oligomer. Since the number of cinnamyl end-groups in lignin is relatively low, lignification in the plant cell wall is believed to be an end-wise polymerization, although there is considerable evidence for cytochemical heterogeneity in lignins. Recently we found that a milled tobacco lignin, like DHPs, has a high content of cinnamyl end-groups,  $\beta$ -5 and  $\beta$ - $\beta$  linkages. The content of end-groups in lignins is therefore an important characteristic of lignin structure. It would be helpful to be able to quantify lignins' end-groups for a better understanding of lignin biosynthesis.

The DFRC (derivatization followed by reductive cleavage) method is a recently developed analytical

tool for lignin characterization (U.S. Dairy Forage Research Center 1996). Through DFRC,  $\beta$ -aryl ether linkages in lignin are cleaved releasing monomers which are quantified by GC. Most monomeric and dimeric DFRC products have been identified. In this study several lignin models with cinnamyl end-groups were subjected to DFRC degradation and major monomers isolated and identified. Mechanisms leading to the formation of these diagnostic monomers are addressed.

## Methods

For GC-mass spectrometric analysis, 5-10 mg substrates were used for DFRC. For preparative scale DFRC, 100-150 mg starting materials were used. AcBr treatment conditions used were standard. AcBr treatment products were separated on normal-phase preparative (2-mm thickness) TLC plates (Alltech, Deerfield, IL) using  $\text{CHCl}_3/\text{EtOAc}$  (20:1) as solvent. The major DFRC final products **3**, **4**, and **7** were isolated from  $\text{C}_{18}$  reverse-phase 1-mm TLC plates (Alltech) using  $\text{MeOH}/\text{water}$ , 6:4, following normal-phase TLC ( $\text{CHCl}_3/\text{EtOAc}$ , 20:1) from preparative DFRC of 4-hydroxycinnamyl alcohols **1** and 4-hydroxycinnamaldehydes **5**.

## Results and Discussion

Coniferyl alcohol **1a**, sinapyl alcohol **1b**, coniferaldehyde **5a**, and sinapaldehyde **5b** were subjected to the DFRC procedure, Fig. 1. Although such units in lignins are completely etherified, reactions on these phenolic models helped elucidate some DFRC pathways; similar reactions with appropriately etherified models, not described here, produced analogous results. Compounds were identified by their mass spectral data, and their structures were authenticated by NMR following isolation.

The major DFRC products from coniferyl alcohol **1a** were 4-acetoxy-guaiacylcyclopropane **3a** and the guaiacylpropyl bromide **2a**. The major monomers from sinapyl alcohol **1b** were the analogous compounds **3b** and **2b**. The unusual cyclopropyl

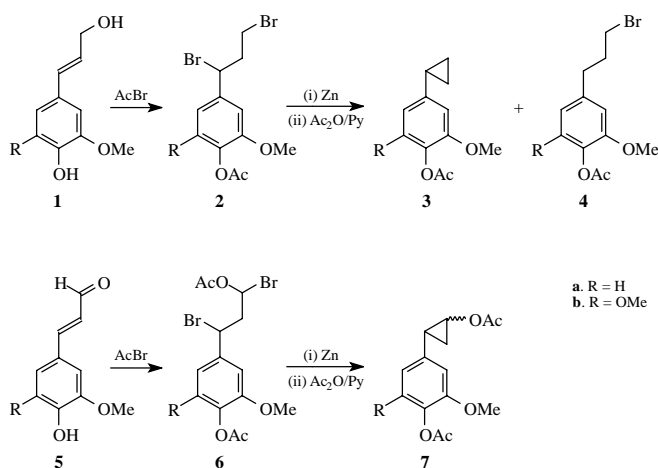


Figure 1. The formation of arylcyclopropane compounds **3** and **7**.

compounds **3** were isolated from preparative DFRC of cinnamyl alcohols **1**. Identification was made by the usual series of NMR experiments ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT, 2D COSY, 2D gradient-enhanced HMQC and HMBC). In the proton NMR spectra of **3**, two multiplet peaks at  $\delta_{\text{H}}$  0.7 and 0.9, integrating for two protons each, indicated the presence of 2 pairs of cyclopropane protons. Those protons correlated with only one carbon peak in HMQC experiments suggesting a symmetry. Carbon-1 of the aromatic ring correlated with all side-chain protons in HMBC experiments, an occurrence not encountered in typical aryl-*n*-propyl side-chains. Thus compounds **3** were identified as arylcyclopropanes.

The major DFRC monomers from coniferaldehyde **5a** and sinapaldehyde **5b** were diagnostic *cis*- and *trans*-aryl cyclopropyl acetates **7**, Fig. 1. Two low-field multiplet signals around  $\delta_{\text{H}}$  1.2-1.3 in the  $^1\text{H}$ -NMR spectra, and the corresponding  $\delta_{\text{C}}$  11.4-11.5 methylene signals in the  $^{13}\text{C}$ -NMR spectra, indicated the presence of cyclopropane protons in compounds **7**. Singlets integrating for 3 protons at  $\delta_{\text{H}}$  1.8-2.0 indicated a side-chain (aliphatic) acetate attached to tertiary carbons. In HMBC experiments, C-1 on the aromatic ring again correlated with all protons on the sidechain, confirming its cyclic nature.

To understand the formation of DFRC monomeric products from cinnamyl alcohols **1** and cinnamaldehydes **5**, the intermediates produced during acetyl bromide (AcBr) treatment were also isolated by

preparative TLC and identified by NMR. 4-Hydroxycinnamyl alcohols **1** resulted primarily in aryl-1,3-dibromopropanes **2**, presumably formed via allylic bromination to the cinnamyl bromide, then HBr addition across the double bond, followed by acetylation. Coniferaldehyde **5a** and sinapaldehyde **5b** reacted with AcBr in acetic acid in a similar way to the alcohols resulting in compounds **6** as major products.

The final compounds are then logical Zn-reductive products of the corresponding intermediate bromides **2** and **6**. Phenylcyclopropane has been obtained by treatment of 1-phenyl-1,3-bromopropane with a Zn-Cu couple in dimethylformamide. Similar ring closure was also observed when comparable reducing conditions were applied to 1,3-dihydraldes. Hence compounds **3** and **7** likely resulted from the 1,3-

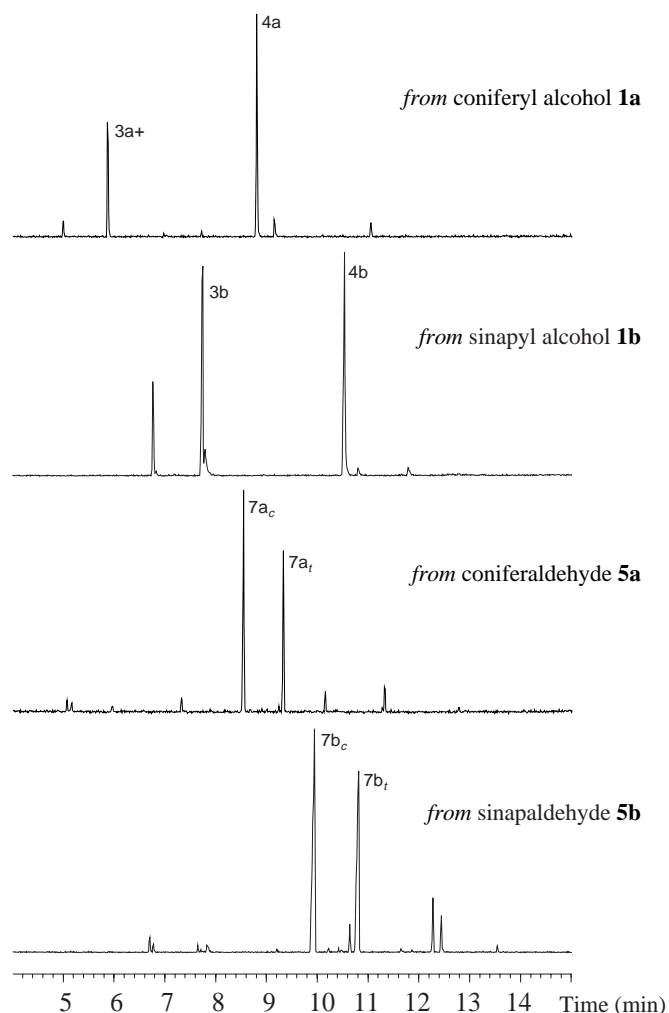


Figure 2. Total-ion chromatograms of DFRC products from cinnamyl and sinapyl alcohols **1a** and **1b**, and cinnamaldehyde **5a** and sinapaldehyde **5b**.

dibromides **2** and **6** formed in the AcBr treatment step. When isolated **2a** was treated with Zn dust in dioxane/acetic acid/water mixed solvent, as in the reductive step of the DFRC procedure, compounds **2a** and **3a** were indeed produced. Compounds **7** were produced analogously when isolated compounds **6** were treated with Zn under DFRC conditions.

## Conclusions

Diagnostic products were formed from cinnamyl alcohol and cinnamaldehyde end-groups in lignins following DFRC treatment. The reactions are not as clean as the ether-cleaving reactions that form the basis of the DFRC method, but nevertheless provide valuable markers for studying end-groups in lignins. Cinnamaldehyde end-groups produce characteristic

arylcyclopropyl acetates, so the DFRC method could find value in understanding compositional changes in mutant and transgenic plants where aldehyde build-up is suspected. Cinnamyl alcohol groups produce 1-aryl-3-bromopropanes, along with more diagnostic arylcyclopropanes and other more minor products. Although the product mixtures are more complex, the production of relatively diagnostic “fingerprint” products from cinnamyl alcohol endgroups also allows the DFRC method to provide useful data on these features of lignins.

## Reference

U.S. Dairy Forage Research Center. 1996. U.S. Dairy Forage Research Center 1996 Research Summaries. U.S. Department of Agriculture, Agricultural Research Service, pp. 62-65.